The Prognostic Value of p53, Bcl-2 and Bax Expression in Laryngeal Cancer

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Objective: *Determine the prognostic value of p53, Bcl-2 and Bax expression in cancer of the larynx.*
Material and Method: *Ninety-four patients diagnosed with laryngeal squamous cell carcinoma were analyzed for 5-year overall survival in relation to immunohistochemical expression of p53, Bcl-2, and Bax proteins.*
Results: *The present study included 86 males and eight females with a mean age of 65.1 years. Half of the patients (51%) were in stages III and IV. Radiation (44.7%) and radiation plus surgery (40.4%) were the main treatments. The frequency of p53, Bcl-2, and Bax expression was 58.1%, 18.5%, and 87.2%, respectively. The 5-year overall survival rate was 49.7%. Univariate analysis revealed that T-stage, N-stage and treatment were significantly associated with 5-year overall survival. In the multivariate Cox regression, T-stage, treatment, and Bcl-2 expression were significantly associated with survival. Positive Bcl-2 expression was associated with better survival (Hazard ratio 0.23, 95% CI 0.06-0.81).*
Conclusion: *The positive Bcl-2 expression is an independent prognostic marker in laryngeal squamous cell carcinoma.*

Keywords: *Larynx cancer, p53, Bcl-2, Bax, Prognostic marker*

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Head and neck cancers are the sixth most common cancers in the world with a global incidence of 700,000 cases per year[1]. Squamous cell carcinoma constitutes the majority of tumor types of head and neck squamous cell carcinoma (HNSCC), including oral cavity, larynx, and pharynx respectively.

In most reports, more than half to two-thirds of the HNSCC patients are at advanced stages of disease at presentation, and this contributes to poor survival of the patients. Even with the advances in medical technologies, the survival outcomes of HNSCC have only subtly increased during the past two decades[2]. Therefore, identification of biological markers to predict a patient's clinical outcome is crucial for effective treatment planning.

Apoptosis and cell cycle control are the two intimately linked molecular pathways involved in carcinogenesis and progression of cancer cells. p53 protein, a product of TP53 tumor suppressor gene, plays a role both in cell cycle control and apoptosis by inducing growth arrest and initiating apoptosis after exposure to DNA damage[3]. Mutation of the TP53 gene results in an abnormal protein that can be detected by a routine immunohistochemical technique. The p53 protein regulates apoptosis via transcriptional activation of Bax and suppression of Bcl-2[4]. Bax and Bcl-2 are important members of the Bcl-2 family proteins, which play roles in the regulation of apoptosis[5]. Apoptotic cell death is an important mechanism for radiation response. Therefore, the ability of tumor cells to confer apoptosis is thought to relate to treatment success or failure.

Many studies have evaluated the prognostic value of p53, Bcl-2, and Bax or other apoptotic proteins in HNSCC, with the largely conflicting results. Therefore, the authors simultaneously evaluated the expression of p53, Bcl-2, Bax in a large series of LSCC.

Material and Method

The present study was reviewed and approved by the institutional ethics committee. The studied subjects included 94 patients who sought treatment at Songklanagarind Hospital, Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Prince of Songkla University, with primary

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laryngeal squamous cell carcinoma (LSCC), from January 2002 to December 2004. The demographic and clinical data were reviewed, as well as the extent of tumor, lymph node involvement, and stage determination, classified according to the International Union against Cancer (UICC) classification, fifth edition, 1997.

Surgical resection or radiotherapy was given for patients in stage I and early stage II cancer, combined surgery and radiotherapy was for late stage II to stage IV disease, and radiochemotherapy for advanced cancer with an unacceptable outcome of surgical morbidity. Radiotherapy alone was considered for palliative treatment in patients with advanced disease who were not physically fit for combined therapy.

Death information was obtained from the census registration data of the Department of Provincial Administration, Ministry of Interior, where the data is linked nationwide. Patients not found dead in this database up to December 2009 were designated as alive in this present study cohort.

Immunohistochemistry and evaluation

Immunohistochemistry was performed on paraffin sections. Antigen retrieval was accomplished by immersing slides in Tris EDTA buffer pH 9 in a pressure cooker at 95°C for 4 minutes. Endogenous peroxidase was blocked by 3% hydrogen peroxide. The slides were incubated with primary antibodies against p53 (clone DO7, DakoCytomation; dilution 1:100), Bcl-2 (clone bcl-2/100/D5, Novocastra; dilution 1:80) and Bax (polyclonal, DakoCytomation; dilution 1:150). The slides were then incubated with EnVision for 30 minutes followed by color development using diaminobenzidine and counterstained with hematoxylin. The sections of esophageal squamous cell carcinoma that were known to be strongly positive for p53 expression were used as positive controls. Sections of endometrial hyperplasia were used as positive controls for Bcl-2 and Bax. Infiltrating lymphocytes were also used as internal positive controls for Bcl-2 and Bax expression.

The percentage of positive stained tumor cells was estimated overall by assessing the whole slide. The presence of more than 5% of Bax and Bcl-2 and 10% of p53 was considered positive expression. Intensity of staining was recorded as weak, moderate and intense.

Statistical analysis

The correlation between clinicopathological variables and protein expression was assessed by Chi-squared test. Five-year overall survival (OS) was obtained by the Kaplan-Meier method. The log-rank test was used to compare differences in survival among subgroups of each variable. Cox proportional hazards regression was performed to obtain independent prognostic factors for survival. The 5% level of significance was considered statistically significant. As no significant difference between using percentage and intensity of protein expression was found, the percentage of expression was used in all analyses. All analyses were carried out using statistical package STATA version 6.0.

Results

The present study included 86 males (91.5%) and eight females (8.5%). The mean age was 65.1 years, with range of 35 to 87. About half of the patients (54.2%) were in advanced stages (stage III and IV) at presentation; among the distribution of stage I/II/III/IV/unknown were 25.5/12.8/23.4/30.8/7.5 respectively. Most of them received radiation (44.7%) or radiation plus surgery (40.4%). However, a number of 10 patients (11%) were lost to follow-up before any treatment was given.

The frequency of p53 expression was 58.1% and most showed strong nuclear staining, and the frequencies of Bcl-2 and Bax expression were 18.5% and 87.2%, respectively with varied intensities from weak to intense.

Median survival time of the patients was 42.7 months. The 5-year overall survival (OS) rate was 49.7% (95% CI 38.8-59.6). Univariate analysis by Kaplan-Meier method and log-rank test revealed that T-stage, N-stage and treatment were significantly associated with 5-year OS. Bcl-2 expression is associated with a higher 5-year OS (72%) compared to a negative Bcl-2 expression (44.4%) with marginal statistical significance ($p = 0.06$), as in Table 1, whereas p53 and Bax expression were not associated with survival.

In the multivariate Cox regression, T-stage, treatment and Bcl-2 remained significant. Bcl-2 expression was independently associated with better survival (Hazard Ratio 0.23, 95% CI 0.06-0.81).

Discussion

The current study showed that Bcl-2 expression was significantly associated with survival, while p53 and Bax expression were not. The several studies showed that p53 expression was not related with the clinical outcomes[6,7], and inconclusive by a meta-analysis[8]. The present study did not show any

Table 1. Univariate and multivariate analyses for clinical parameters and protein expression in relation to overall survival (n = 94)

Variables	5-yr overall survival (%)	p-value[a]	Hazard ratio	95% CI	p-value
T stage					
T1	74.7	0.01	1		
T2	48.0		4.67	1.49-14.59	0.01
T3-4	36.9		9.14	3.06-27.35	0.00
N stage					
N0	58.2	0.03	1		
N1	37.5		0.87	0.27-2.77	0.83
N2-3	33.3		0.91	0.40-2.06	0.82
Treatment					
Untreated	0	0.00	1		
Surgery	66.7		0.14	0.01-1.27	0.08
Radiation	47.92		0.25	0.09-0.73	0.01
Surgery + radiation	61.8		0.08	0.02-0.27	0.00
P53 expression					
Negative	44.2	0.59			
Positive	54.6		0.65	0.32-1.32	0.24
Bcl-2 expression					
Negative	44.4	0.06			
Positive	72.1		0.23	0.06-0.81	0.02
Bax expression					
Negative	58.3	0.52	1		
Positive	48.0		1.07	0.33-3.54	0.91

[a] log-rank test

statistical significance of Bax expression, consistent with other studies[9,10].

Bcl-2 contributes to neoplastic cell expansion by preventing cell turnover caused by the physiological cell death mechanism. Over-expression of the Bcl-2 also prevents cell death induced by nearly all cytotoxic anticancer drugs and radiation[11]. Most of these studies reported a lack of association of Bcl-2 expression with clinical outcomes; however, a few studies demonstrated a positive association[12,13] or even a reverse association (Bcl-2 expression associated with better prognosis)[14,15], as the present study. Some authors have demonstrated lower Ki-67 labeling index and/or apoptotic labeling index in Bcl-2+ foci/tumor and these tumors are associated with a better prognosis[16,17].

In conclusion, the positive Bcl-2 expression is an independent prognostic marker in laryngeal squamous cell carcinoma.

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Potential conflicts of interest

None.

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คุณค่าการพยากรณ์ของการแสดงออกโปรตีน p53, Bcl-2 และ Bax ในมะเร็งกล่องเสียง

ปลื้มจิต บุณยพิพัฒน์, โกวิทย์ พฤกษานุศักดิ์, ปารมี ทองสุกใส

วัตถุประสงค์: *เพื่อระบุหาคุณค่าการพยากรณ์ของการแสดงออกโปรตีน p53, Bcl-2 และ Bax ในมะเร็งกล่องเสียง*
วัสดุและวิธีการ: *ผู้ป่วยจำนวน 94 ราย ผู้เป็นมะเร็งกล่องเสียงชนิดเซลล์แบน ได้วิเคราะห์การอยู่รอดภาพรวม 5 ปี มีความสัมพันธ์กับการแสดงออกของโปรตีน p53, Bcl-2 และ Bax ด้วยวิธี immunohistochemistry*
ผลการศึกษา: *การศึกษานี้มีผู้ป่วยเป็นชาย 86 ราย และหญิง 8 ราย อายุเฉลี่ย 65.1 ปี ผู้ป่วยครึ่งหนึ่ง (ร้อยละ 51) เป็นระยะที่สามและสี่ การรักษาโดยวิธีรังสีรักษา (ร้อยละ 44.7) และรังสีรักษากับการผ่าตัด (ร้อยละ 40.4) เป็นการรักษาหลัก อัตราการรอดชีวิตภาพรวม 5 ปี เท่ากับร้อยละ 49.7 ความชุกของการแสดงออกของโปรตีน p53, Bcl-2 และ Bax เท่ากับร้อยละ 58.1, 18.5 และ 87.2 ตามลำดับ พบว่าการรอดชีวิตภาพรวม 5 ปี มีความสัมพันธ์อย่างมีนัยสำคัญกับระยะขนาดก้อนมะเร็ง ระยะต่อมน้ำเหลือง และการรักษา ด้วยการวิเคราะห์ตัวแปรเดี่ยว เช่นเดียวกับ ระยะขนาดก้อนมะเร็ง การรักษา และการแสดงออก Bcl-2 ด้วยการวิเคราะห์พหุตัวแปรแบบ Cox การแสดงออก Bcl-2 ได้ผลบวกสัมพันธ์กับพยากรณ์โรคที่ดี (Hazard ratio 0.23, 95% CI 0.06-0.81)*
สรุป: *การแสดงออกของโปรตีน Bcl-2 ได้ผลบวกเป็นปัจจัยพยากรณ์ของมะเร็งกล่องเสียง*